On-chip digital droplet PCR quantification of miRNA using a multiplexing assay

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Recent advances show that miRNA has a great potential in diagnostics. Several studies demonstrate that the expression profile of miRNAs, which are short (18-22 nucleotides) but powerful regulators of a wide range of biological processes, can aid in distinguishing between healthy and diseased patients. As these regulators are detectable in peripheral blood, they can perfectly serve as blood-based biomarkers. An interesting way to profile miRNAs involves the use of lab-on-chip tools, which allow automated quantification. Therefore, it is of interest to implement the detection of miRNA into a microfluidic device or chip. As digital droplet PCR (ddPCR) has shown to be more accurate and has an improved reproducibility compared to regular qPCR, microfluidic chips (9x9 mm) for ddPCR were fabricated using in house silicon technology. Preliminary results show that the simultaneous detection of two miRNAs (Let-7a and miR-39) in picoliter-sized droplets using this design is possible. Current efforts are ongoing to further increase the multiplex capability as well as to simplify miRNA detection by combining the RT and ddPCR step in one droplet. This will lead to an optimal usage of this chip configuration.